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O. B. B. B.

Application No. 1263

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Bruce, Wesley B. Date: March 17, 2003
Serial No.: 09/970,624 Group Art Unit: 1638
Filed: October 4, 2001 Examiner: A. Mehta
For: "A Nitrate Responsive Root Transcriptional Factor"

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF WESLEY B. BRUCE UNDER 37 CFR § 1.132

I, Wesley B. Bruce, declare:

I am a citizen of the United States of America and a resident of Urbandale, Iowa, United States of America.

I received a degree of Doctor of Philosophy in May 1987, in the area of Plant Molecular Biology from the University of Florida, in Gainesville, Florida.

I received a degree of Bachelor's of Science in Microbiology and Cell Science, in August 1981, from the University of Florida, in Gainesville, Florida.

I presently hold the position of Research Scientist at Pioneer Hi-Bred International, Inc. from August 1991- present. Research areas include: functional genomics, gene expression, and plant development.

I was employed as an Adjunct Professor in the area of Plant Molecular Biology at Northern Illinois University, DeKalb Illinois, in 1991. My research focus was on developing ribozyme technology for engineering novel traits in plants.

I was a Postdoctoral Fellow in Plant Molecular Biology at Northern Illinois University, DeKalb Illinois, in 1990. My research focus was again on the developing ribozyme technology for engineering novel traits in plants.

I was a Postdoctoral Fellow in Plant Molecular Biology at The Plant Gene Expression Center/University of California at Berkeley in Albany, California from 1987 -

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1990. My research focus was on characterizing light-regulated gene expression through phytochrome signal-transduction pathways.

I am personally familiar with the method of collection of the data provided below.


My research indicates that comparing the sequence identity of the MADS2 transcription factor to the disclosed sequences is not enough evidence to conclude that the claimed SEQ ID NO: 1 does not function as a root transcriptional factor polynucleotide. SEQ ID NO: 1 has been determined to be expressed primarily in roots based on the Massively Parallel Signature Sequencing (MPSS) Expression Analysis of Lynx Therapeutics, Inc., 25861 Industrial Blvd., Hayward, CA. This MPSS Expression Analysis is a very sensitive and reproducible means of measuring the expression levels of thousands of genes from a variety of plant tissues. The SEQ ID NO: 1 was detected at significant levels in multiple root tissue samples including lateral roots. The expression pattern of this polynucleotide is represented in 58% of the proprietary maize root cDNA libraries. Whereas this polynucleotide was expressed in only 6% of the maize tassel cDNA libraries and was undetectable in any of the proprietary maize pollen cDNA libraries. These results support that the SEQ ID NO: 1 transcriptional factor polynucleotide is primarily expressed in root tissues, and therefore does not solely fit a pollen-specific expression pattern. Also, from greater than 600,000 proprietary ESTs, the Arabidopsis ANR1 sequence showed a significant match to the SEQ ID NO: 1 based on my comparative sequence searches. These proprietary ESTs include a substantial representation of sequences from tassel- and root-expressed genes. Based on the foregoing, I disagree with the Examiner's statement that the specification discusses "a signal transduction pathway that links external nitrate to increase lateral root proliferation and that manipulation of nitrate-responsive genes such as ANR1 in agronomic crops could be of value in maximizing plant utilization of available nitrogen and in reducing agricultural nitrogen inputs, that improved control of lateral root

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proliferation could have useful applications in soil remediation, that increased root biomass may be beneficial in production of specific structural carbohydrates, and that manipulation of nitrate-responsive genes could also be useful in stimulating root proliferation of cuttings for plant propagation", and I assert that the MPSS data demonstrates that this polynucleotide sequence is functioning in root tissue. The sequence similarity to ANR1 and the expression distribution across the proprietary maize libraries demonstrate that SEQ ID NO: 1 is functioning primarily in maize roots, including the maize lateral root tissues, and minimally in tassel-related tissues.

Based on this information it is my conclusion that the polynucleotide disclosed and claimed as SEQ ID NO: 1 functions as a nitrate-responsive transcription factor in maize root systems. Therefore the present invention has a well-established utility for which has been proposed a specific, substantial and credible use.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.


Wesley B. Bruce

Dated: 3-17-03